Oxygen and brain death; back from the brink

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New Findings

What is the topic of this review?

To explore the unique evolutionary origins of the human brain and critically appraise its energy budget including limits of oxygen and glucose deprivation during anoxia and ischaemia.

What advances does it highlight?

The brain appears to be more resilient to substrate depletion than traditionally thought highlighting greater resilience and an underappreciated capacity for functional recovery.

Abstract

The human brain has evolved into an unusually large, complex and metabolically expensive organ that relies entirely on a continuous supply of O_2 and glucose. It has traditionally been assumed that its exorbitant energy budget combined with little to no energy reserves renders it especially vulnerable to anoxia and ischaemia with substrate depletion and progression towards cell death largely irreversible and rapid. However, new and exciting evidence suggests that neurons can survive for longer than previously thought highlighting an unexpected resilience and underappreciated capacity for functional recovery that has changed the way we think about brain cell death. Nature has the potential to unlock some of the mysteries underlying ischaemic survival with select vertebrates having solved the problem of anoxia-hypoxia tolerance over millions of years of evolution. Better understanding of their survival strategies including remarkable adaptations in brain physiology and redox homeostasis, may help identify new therapeutic targets for human diseases characterised by O_2 deprivation, ischaemic/reperfusion injury and ageing.

Introduction

Oxygenic photosynthesis preceded the inexorable rise in oxygen (O_2) in the atmosphere and surface oceans ~2.4 billion years ago during the Great Oxidation Event in a world dominated by Bacteria and Archaea (Holland, 2006). This was the most important event in the evolution of life during Earth's 4.5 billion years history, with each O_2 "pulse" linked inextricably to major evolutionary and developmental innovations including the emergence of aerobic respiration with adenosine triphosphate (ATP) the universal energy source and cephalisation, a characteristic feature inherited from the last common bilaterian ancestor that led to the first appearance of a central nervous system (CNS) and ultimately, the vertebrate brain (Bailey, 2019b).

Through impressive elaboration of its molecular "toolkit", the modern human brain has since evolved to become the most complex structure in Nature yet remains shrouded in mystery; unusually large, anatomically complex and bioenergetically expensive to maintain, strangely constrained by next to no fuel stores and as a consequence, especially vulnerable to failure when its blood supply is cut off for just a few minutes; or so it was thought. The current review explores the unique evolutionary origins of the human brain and highlights its bioenergetic limits. New and exciting evidence from landmark experiments suggest that neurons can somehow survive ischaemia far longer than previously thought challenging the long-held assumption that the mammalian brain is irreversibly damaged soon after blood stops circulating. This has revealed an unexpected resilience and underappreciated capacity for functional recovery, an exciting prospect that has sparked widespread and oftentimes heated debate.

Human tolerance will also be compared and contrasted against some of nature's "extreme" vertebrates, select species that have solved the problem of anoxia-hypoxia tolerance with specialist adaptations in brain physiology and redox homeostasis honed over millions of years of evolution. Better understanding of their survival strategies that allow them to endure levels of hypoxaemia that would be otherwise fatal to (mere-mortal) human life may help unlock some of the mysteries of the human brain and identify new therapeutic targets for diseases characterised by O_2 deprivation, ischaemic/reperfusion injury and ageing.

Origins and evolution of the human brain; burgeoning energy budget

The human brain lies at the very heart of what makes us human, yet the origin and evolutionary history of the nervous system, its centralisation and subsequent emergence of the brain from an invertebrate chordate ancestor remains obscure (Martin-Duran *et al.*, 2018). Thought to have

occurred before the Palaeozoic era ~540 million years ago (Mya), the prevailing consensus is that an ancestral bilaterian possessed a bipartite brain consisting of a diencephalic forebrain–midbrain region and hindbrain–spinal cord region and migratory ectodermal sensory cells directed towards the management of feeding, digestion, movement and orientation. Further elaborations of its molecular "toolkit" including whole genome duplications in vertebrates allowed more genes to be added to existing gene networks facilitating the evolution of new and more complex structures such as the neural crest, placodes and midbrain-hindbrain organiser (Holland, 2009).

How the human brain subsequently evolved from hominids living ~3-5 Mya is comparatively less controversial given a more complete fossil record culminating in what we understand the modern human brain represents today; an unusually large, complex, globular, slow to mature, high-energy consuming organ! Indeed, the brain has more than tripled in size over the last ~3 Mya since *Australopithecus* (~400-600 cm^3 to 1,200-1,600 cm^3) with humans the most encephalised of all species boasting a brain that is seven times larger than expected for our body mass courtesy of a dramatically enlarged neocortex (Figure 1A) (Schoenemann, 2006). This has been accompanied by a proportional increase in neurons (~29 to 96 × 10^9, Figure 1B) (Azevedo *et al.*, 2009), a consequence of increased symmetric neural progenitor cell divisions in the ventricular zone (Roth & Dicke, 2005) with even greater increases (~6-fold,) observed in cerebral perfusion (Figure 1C) (Seymour *et al.*, 2017) and glucose consumption (Figure 1 D) (Herculano-Houzel, 2011) needed to fuel rapid developments in processing power including enhanced interneuron connectivity, synaptic activity, complex movements and cognitive function (Semendeferi *et al.*, 2002; Schoenemann, 2006; Azevedo *et al.*, 2009; Seymour *et al.*, 2016).

However, being big comes at a cost; neural tissue is especially expensive to maintain, up to 16 times more costly than skeletal muscle and other somatic tissues (Isler & van Schaik, 2006). This is not

surprising given its anatomical complexities (Figure 2A) and computational (neural processing) capabilities (Figure 2B). The latter is estimated to be in the order of ~1 exaFLOP (Waldrop, 2012), equivalent to a quintillion (10^{18}) floating point operations per second (flops, a measure of the numerical computing performance of a computer). This is far superior to even the most advanced super computers ultimately constrained by von Neumann architecture in which memory is separate from the central processing unit.

Yet in spite of its meagre energy stores and weighing less than $1/50^{th}$ of the total body mass, the human brain allocates a disproportionate 20–25% of the basal energy budget (Kety, 1957) which is further prioritised during childhood rising to a lifetime peak of 66% (Kuzawa *et al.*, 2014) to fuel resting maintenance of ionic equilibria and uptake of neurotransmitters for synaptic transmission; so-called "dark energy" analogous to "dark matter" in physics parlance (Raichle, 2006), turning over a staggering 8.3 kg of ATP/day, equivalent to six times the brain's very own mass (Zhu *et al.*, 2018) helping put its exorbitant energy needs into clearer perspective (Figure 2C). This is far in excess of the 8–10% allocation observed in non-human primates and 3–5% in most non-primate mammals (Leonard *et al.*, 2003) ultimately limiting the maximum rate at which the brain can compute with neural information processing in the millisecond range analogous to a "clock speed" of ~1 kHz (compared to 3 GHz for most super computers) despite evolutionary pressure for metabolically efficient wiring patterns and neural codes to optimise signal transmission efficiency (Attwell & Gibb, 2005); and all simply because humans have more neurons to nourish, in fact, a whopping ~86 x 10^9 of them! (Herculano-Houzel, 2011).

Precisely why such remarkable brain expansion was favoured within the genus *Homo* remains unclear though clearly under strong, if not indeed accelerated selection with the (cognitive) benefits outweighing (metabolic) costs, consistent with adaptive evolution (Dorus *et al.*, 2004). Improved diet

quality, allomaternal subsidies, cognitive buffering, reduced locomotion costs and allocation to production are all factors that likely contributed to this evolutionary trade-off (Navarrete *et al.*, 2011). As the highest regulatory authority, the brain needs to look after itself first, prioritising energy needs above all other organs, consistent with the "Selfish Brain Hypothesis" (Peters *et al.*, 2004). Indeed, the overarching drive of mammalian physiology has involved this hierarchal order of operations (brain over brawn) with expansion also observed in lower mammals including the proboscidea (elephants), cetaceans (whales and dolphins) and select species of pinnipeds (seals). Like *Homo*, their brains were catapulted beyond the 700 g "barrier" in mammalian brain mass evolution (Manger *et al.*, 2013) suggesting that the well-conserved biology was likely a prerequisite for human encephalisation and development.

Yet it's where you put your neurons that ultimately counts, cognitively. The brain of the African elephant, *Loxodonta africana* is the largest of any living land-dwelling mammal (4.619 kg), jam-packed with ~257 x 10^9 neurons which is three times more than the average human. Yet unlike humans, ~98 % of its neurons are located in the cerebellum and not the cortex (Herculano-Houzel *et al.*, 2014). However, it would seem that expansion has come to the end of the road with the human brain undergoing considerable "downsizing" (~240 mL) during the Holocene (past 10,000 years) equivalent to 36 times the rate of increase observed over the past 800,000 years (Henneberg, 1988), suggesting that we will never fulfil our processing capacity potential, with the upper limit estimated at ~3,500 cm^3 (Hofman, 2014).

Pushing the limits; the brain's bioenergetic tightrope

Unable to compromise on such an exorbitant energy budget combined with little to no O_2 or glycogen reserves renders the human brain especially susceptible to anoxia and ischaemia with

cerebral microvascular architectonics following an evolutionary path more focused on optimising O_2 supply than ($O_{2)}$ reserve) (Hadjistassou *et al.*, 2015). Indeed, unless cerebral perfusion is rapidly restored, failure of ATP-dependent ion exchangers results in the breakdown of ionic gradients and membrane depolarisation, triggering a cytotoxic increase in intracellular Ca^{2+} concentration and uncontrolled release of excitatory neurotransmitters that ultimately converge in progressive and for the most part irreversible, neuronal death (Lipton, 1999). In support, cerebral ischaemia has been shown to trigger neuronal death within only 5 minutes compared to a substantially longer period of 20–40 minutes in cardiac myocytes and kidney cells (Lee *et al.*, 2000).

This unique sensitivity to ischaemia was highlighted during a controversial series of human experiments known as the Red Wing studies. Entitled “Acute arrest of circulation in the human brain”, the investigators took advantage of non-consenting psychiatric patients to better understand why pilots were blacking out and to assess “anoxic shock therapy” as an alternative treatment for schizophrenia (Rossen *et al.*, 1943). Following application of a specialised cervical pressure cuff that could be inflated to 600 mmHg within $^{1}/_{8th}$ s, unconsciousness was consistently observed within 4-10 s of ischaemia that was further extended up to a staggering 100 s (Figure 3A).

Simple division of the brain’s metabolic rate(s) by energy content (Figure 2B) provides a rough estimate of its bioenergetic limits, put simply how quickly the meagre fuel reserves would be “emptied” if ischaemia was further prolonged. The first “fuel” to suffer is oxygen (O_2), with limited reserves depleted in a single second followed swiftly by glucose. Note the “critical” arterial concentrations that serve as warning signs of impending doom, below which substrate depletion predisposes to unconsciousness, coma and ultimately, neuronal damage (based on data cited in (Cryer, 2007; Bailey *et al.*, 2017) (Figure 3A).

Three less controversial yet equally unique research studies have helped identify "tolerable" limits of arterial hypoxaemia and associated convective/diffusive components of the cerebral "O_2 cascade" in the healthy human (Figure 3B). The lowest PaO_2 (16 mmHg, gold award) was recorded during nitrogen hyperventilation (anoxia) though this ultimately led to unconsciousness within 17-20 s (Ernsting, 1963). Equivalent (albeit marginally less severe) hypoxaemia was documented in a mountaineer exposed to the hypocapnic hypoxia of extreme terrestrial high-altitude on Mt. Everest (PaO_2 of 19 mmHg, $PaCO_2$ of 16 mmHg, silver award (Grocott *et al.*, 2009)) and in a hypercapnic freediver following static apnoea (PaO_2 of 23 mmHg, $PaCO_2$ of 61 mmHg, bronze award (Bailey *et al.*, 2017), implying that these athletes were operating close to, if not indeed at, the very limit of human consciousness.

Ischaemic preconditioning (IP) could in theory extend these limits through induction of a more resilient phenotype (Nyquist & Georgakis, 2019). This has been shown to confer neuroprotection in surgical patients (Dirnagl *et al.*, 2009) and contributes to the extraordinary anoxia-ischaemia tolerance exhibited by some of nature's "extreme" vertebrates in whom the mechanisms needed for anoxic survival are already in place, including the "constitutively preconditioned" freshwater turtle, *Trachemys scripta* and crucian carp, *Carassius carassius*, species that are essentially immune from brain damage (Nilsson & Lutz, 2004) (see later). It is not by chance that the evolution and ongoing survival of the human brain, including the mechanisms shared by IP and anoxia tolerance have a hormetic basis, unified by the adaptive formation of free radicals (Milton & Prentice, 2007; Bailey, 2019b). Select species including the superoxide anion have the thermodynamic capacity to exploit "quantum fast" signalling to preserve O_2 homeostasis, an evolutionary conserved response that was already being exploited by the last universal common ancestor ~3.8 billion years ago (Bailey, 2019b).

However, to what extent repeated and prolonged exposure to these physiological extremes of hypoxaemia, alkalosis, and acidosis has an adverse impact on the brain remains an open question, though emergent MRI and immunochemical data suggest an association with structural brain damage and long-term cognitive complications (Andersson *et al.*, 2009; Bailey *et al.*, 2009; Gren *et al.*, 2016). This has been linked to disrupted redox homeostasis characterised by excessive free radical formation, reflecting a shift from a physiologically adaptive (hormetic) to a pathologically maladaptive (damaged) phenotype (Bailey *et al.*, 2013; Bailey *et al.*, 2019).

Beyond limits; the brain's unexpected resilience

However, new findings have provided a veritable "twist in the tale", challenging the long-held assumption that the mammalian brain is irreversibly damaged soon after blood stops circulating. This has highlighted hitherto hidden energy reserves, a built-in insurance policy that has forced a reappraisal of our current understanding of the brain's bioenergetic limits. Neuronal, electrophysiological and metabolic recovery has been observed following 1h of global experimental ischaemia in the macaque brain (Hossmann & Zimmermann, 1974) and up to 6 h following cardiac arrest in rodents (Charpak & Audinat, 1998). A more recent landmark study identified that select aspects of molecular, haemodynamic and structural function could be maintained in the pig brain for up to 6 h after a 4 h postmortem (PM) interval using a customised isolated perfusion system (Figure 4A) that included a remarkable resurgence in global cerebral metabolism and retained dilatory functionality to pharmacological stimulation (Vrselja *et al.*, 2019).

In humans, mitochondria isolated from the cerebral cortex has been shown to remain functional for up to 10 h PM and can be maintained in culture for as long as 78 days (Verwer *et al.*, 2002). Favourable patient outcomes have been reported following endovascular thrombectomy performed

up to 16 h after ischaemic stroke (Albers *et al.*, 2018). Furthermore, therapeutic hypothermia during elective surgery is considered neuroprotective buying surgeons and patients precious time with accidental hypothermic cardio-circulatory arrest induced by cold exposure/drowning shown to extend the brain's "survival" time by up to as much as 9 h in the most extreme cases without any evidence of neurological damage (Figure 4B) (Gilbert *et al.*, 2000; Forti *et al.*, 2019).

A recent study in patients suffering from devastating brain injuries in whom life-sustaining therapy was withdrawn while neuromonitoring continued as they died is especially revealing (Dreier *et al.*, 2018). Neurons were shown to shift into an energy-saving standby mode after 20-40 s of O_2 deprivation; electrical activity stopped, the brain flatlined and became "silent" (termed non-spreading depression), that would traditionally have been taken to reflect irreversible progression towards neuronal death. However, the brain "lingered" in this supressed state for a further ~3 minutes before its fuel reserves became fully depleted. Subsequent collapse of ionic gradients resulted in sudden bursts or waves of (stored) electrochemical energy akin to the charge released from discharging batteries, known as spreading depolarisations or "brain tsunamis" that ripple through the brain further compounding ischaemia. These can be endured for a further ~3->10 minutes during which time the loss of polarisation is potentially reversible and neuronal integrity recoverable if the fuel supply (i.e. perfusion) can be restored ahead of an all-important "commitment point". Beyond this, a final surge of energy (terminal spreading depolarisation, see Figure 4B inset) reflects the moment brain death is thought to finally become irreversible.

Collectively, these findings indicate that neurons can somehow survive ischaemia longer than traditionally assumed though precisely what, where and how these additional energy stores can be accessed in the human brain remains to be elucidated. However, we don't know whether all neurons can survive ischaemia, or if survival extends to glial cells. Furthermore, to what extent the neuronal

ensembles that interpret the world around us remain functional during recovery from the ischaemic challenge is equally unclear since living neurons don't necessarily equate to a “functional brain" defined by awareness, perception, or other higher-order brain processes (Vrselja *et al.*, 2019). These unknowns have sparked widespread debate among clinicians, ethicists and society at large, questioning current interpretation/clinical diagnosis of brain death and the complex decision-making underpinning resuscitation and organ transplantation (Farahany *et al.*, 2019; Youngner & Hyun, 2019).

Building a better brain; lessons from nature

Though impressive, the human brain’s bio-energetic limits, both known and hitherto unexplored, pale into insignificance compared to some of nature’s first division of anoxia tolerant vertebrates, the true masters of adaptation (platinum awards). These species exist as swimmers, divers, burrowers, hibernators and flyers, equipped and are equipped with remarkable specialisations of brain physiology honed over millions of years of evolution (Figure 5). The most tolerant of the teleosts, the crucian carp, *Carassius carassius*, can survive up to 5 months of anoxia while entombed beneath winter ice without neuronal integrity being compromised, limited only by exhaustion of its enormous liver glycogen stores (Nilsson, 2001). Likewise, the diving seal, *Cystophora cristata* can endure PaO_2’s as low as 7 mmHg with brain function fully preserved (Elsner et al., 1970). Equally fascinating, the “high-fliers” including Ruppell’s griffon, *Gyps rueppellii* and the bar-headed goose, *Anser indicus* have been observed at altitudes of 9,000–11,278 m with the former capable of achieving the high metabolic rates required for flapping flight and running even when exposed to a simulated PO_2 equivalent to that encountered on the summit of Mt. Everest (Hawkes *et al.*, 2014).

The remarkable hypoxaemia endured by these specialists continues to puzzle physiologists, oftentimes exceeding mathematical predictions of O_2 store depletion.

Each species is endowed with unique specialist adaptations in brain (notwithstanding cardiopulmonary) physiology and (aforementioned) constitutive redox defences that allow them to survive such seemingly impossible feats (Figure 5). The "best-of-the-best", the crucian carp and freshwater turtle, achieve this by flipping their brains into "standby" mode, a reversible coma or state of metabolic suppression that effectively shuts down ion flux/neurotransmitter release and by protecting neurons from free radical-mediated reperfusion/hyperoxygenation injury during recovery (Nilsson & Lutz, 2004; Nayak *et al.*, 2016).

Metabolic suppression accompanied by ion channel arrest and reduction in body temperature, cerebral perfusion and neuronal activity is also observed during the dramatic and prolonged state of energy conservation characterising hibernation (Carey *et al.*, 2003). When the arctic ground squirrel, *Urocitellus parryii* transitions from euthermia to the torpid state of "suspended animation", its metabolic rate decreases to an astonishing 1-2 % of basal levels and CBF is reduced by up to as much as 10-fold (Frerichs *et al.*, 1994). Yet it emerges unscathed without any evidence of brain injury even during the course of cerebral reperfusion, exhibiting, to the contrary, signs of accelerated restoration including synaptogenesis (von der Ohe *et al.*, 2006) and cognitive enhancement (Weltzin *et al.*, 2006).

Originally thought to be restricted to cold-adapted mammals, hibernation has also been observed in primates (dwarf lemurs) (Dausmann *et al.*, 2004; Blanco *et al.*, 2013) implying that perhaps we humans have the ability to hibernate which has beneficial applications in medicine and long-term space exploration (Chouker *et al.*, 2019). Central metabolic roles for pyruvate dehydrogenase kinase isoenzyme 4 (Faherty *et al.*, 2018) and hibernation-specific protein (HP20 complex) (Kondo *et al.*,

2006) have been suggested as ancestral "hibernation signatures" that contribute to mammalian brain signalling and neuroprotection.

Conclusions and future directions

The human brain has evolved into an unusually large, complex and metabolically expensive organ that relies entirely on a continuous supply of O_2 and glucose. It has traditionally been assumed that an excessive energy budget combined with little to no energy reserves renders it especially vulnerable to anoxia and ischaemia with substrate depletion and progression towards neuronal death largely irreversible and rapid. However, new and exciting evidence suggests that neurons can somehow survive far longer highlighting an unexpected resilience and underappreciated capacity for functional recovery that has changed the way we think about brain cell death. While impressive, human tolerance still falls well short of nature's more resilient "extremophiles", specialist vertebrates that can survive anoxia courtesy of remarkable adaptations in brain physiology and redox homeostasis that have been shaped over millions of years of evolution. Better understanding of the neuroprotective strategies exploited by these specialists may help unlock some of the mysteries of the human brain including its "hidden" O_2 stores/resilience. This may help identify new therapeutic targets for diseases characterised by O_2 deprivation, ischaemic/reperfusion injury and ageing (Nilsson & Lutz, 2004; Krivoruchko & Storey, 2010; Milton & Dawson-Scully, 2013; Larson *et al.*, 2014).

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Additional information

Competing interests

None declared.

Author contribution

DMB conceived and wrote the manuscript. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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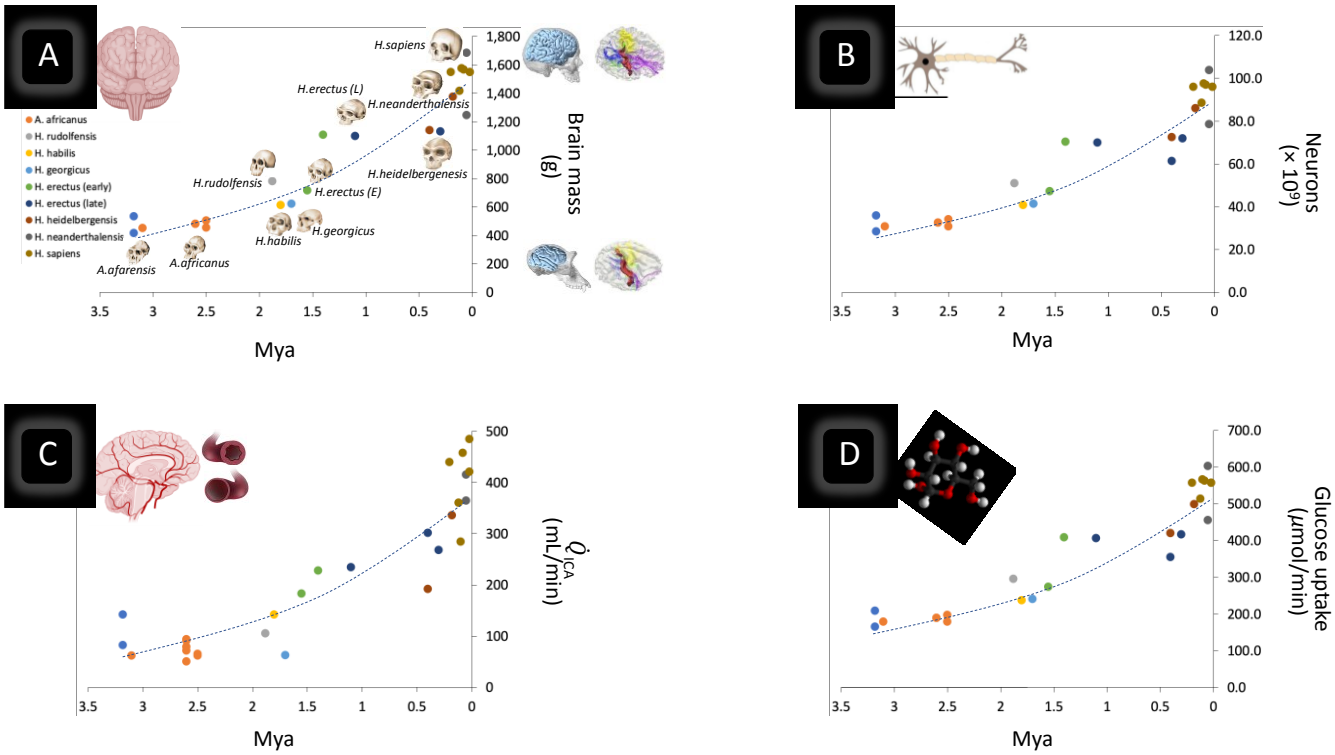

Figure 1. Evolutionary drive for brain size; bigger, bulgier and better

A. Brain mass derived from (corrected) endocranial volumetric data cited in (Seymour *et al.*, 2017) assuming brain tissue density of 1.036 g/cm^3 (Stephan *et al.*, 1981). Compared to extant primates such as the common chimpanzee (*Pan troglodytes*), one of our closest living relatives from whom we descended ~5 million years ago (Mya) and share ~96 % of our DNA (Chimpanzee & Analysis, 2005), the human brain is dramatically enlarged (especially the neocortex and cerebellum), bulgier and better connected. Diffusion tensor images taken from (Zhang *et al.*, 2013) with fibres connecting to the frontal lobes coloured purple, blue for occipital lobes, yellow for the other hemisphere and green for subcortical regions). B. Neuron count calculated from the exponent, brain mass$^{0.923}$ × 109,239,790.169 (Herculano-Houzel & Kaas, 2011). C. Cerebral blood flow given as internal carotid artery flow ($\dot{Q}_{ICA}$) derived from dimensions of the carotid foramina and converted to mL/min, modified from corrected data cited in (Seymour *et al.*, 2017). D. Glucose uptake given by 0.0058 × neuron count in millions (from B) based on linear interpolation of data reported by (Herculano-Houzel, 2011). All relationships best described by an exponential function (r^2 = 0.825-0.927, P < 0.05).

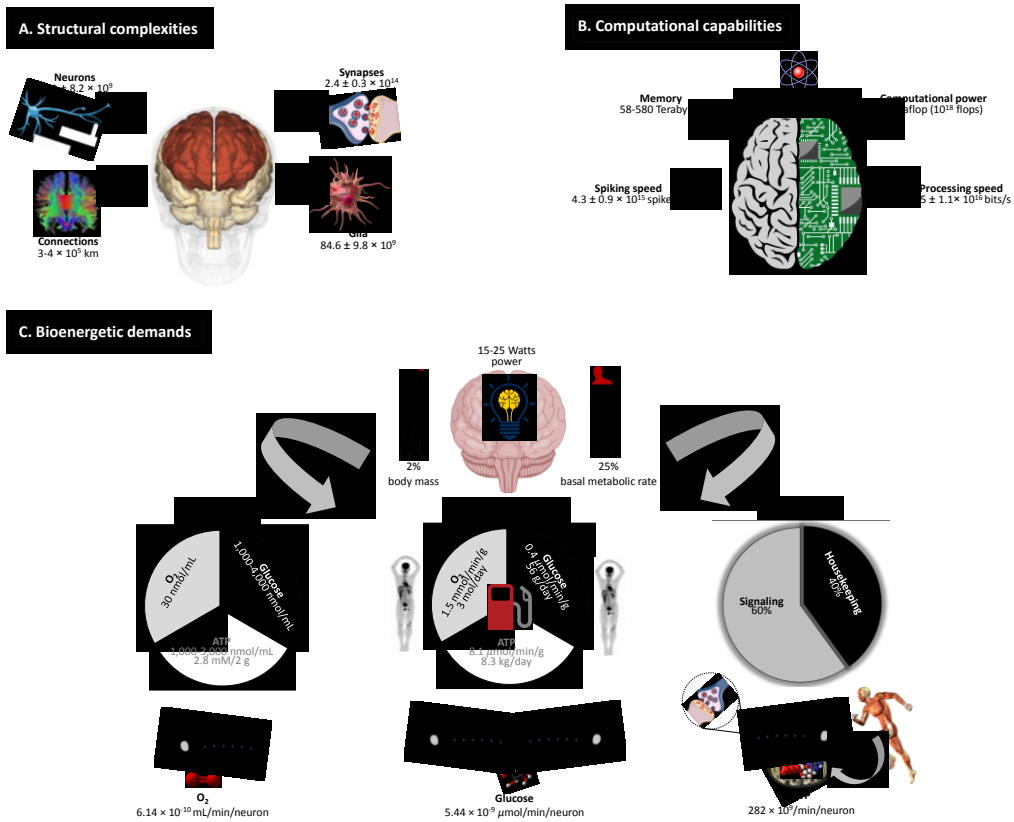

Figure 2. Fuel for thought; human brain's energy budget

Growing and running the human brain is energetically expensive. Structural complexities computational capabilities and bioenergetic demands reflect whole brain estimates based on data cited in (Kety, 1957; Azevedo *et al.*, 2009; Herculano-Houzel, 2011; Waldrop, 2012; Engl & Attwell, 2015; Cherry *et al.*, 2018; Bailey, 2019b; Martins *et al.*, 2019). A-B. It has been estimated that given its structural complexities, the computational power required to simulate human brain function (such as the Blue Brain simulation, a prototype for the Human Brain Project) needs to fall within the exaflop range, equivalent to 10^{18} floating point operations per second (flops) (Waldrop, 2012). Currently, the world's fastest computer (Summit, Oak Ridge National Laboratory, TN, USA) operates within the petaflop range (200 x 10^{15} flop/s) with the first exascale computer eagerly anticipated for

2021. An emergent field, termed “physics-iology”, argues that energy/information transfer in the human brain may well (have to) exploit the notoriously counterintuitive behaviours underlying quantum mechanics, including such “tricks” as superposition, tunnelling, entanglement and altered coherence, moving beyond traditional (i.e. classical) approaches in order to explore deeper meanings and unravel more precisely how our brain functions (Bailey, 2019a). C. Summary of the human brain’s bioenergetic demands, highlighting its limited energy reserves in the form of oxygen (O_2), glucose and adenosine triphosphate (ATP) combined with high rates of metabolism. Note the almost exclusive cerebral uptake of glucose as illustrated by the (first) whole body 3D PET/CT image using ^{18}F-fluorodeoxyglucose (Cherry *et al.*, 2018) and extraordinarily high rates of neuronal metabolism [numbers reflect average energy consumption/turnover based on an average brain mass of 1508.91 g and 86.06×10^9 neurons documented in (Herculano-Houzel, 2011)]. Indeed the cerebral rate of ATP turnover at rest alone is so high it is roughly equivalent to that documented in human skeletal (leg) muscle during the extreme physical demands of a marathon (Hochachka, 1994). Given such a high metabolic rate, action potentials have been rendered highly efficient through evolution with most of the energy consumed to support synaptic activity.

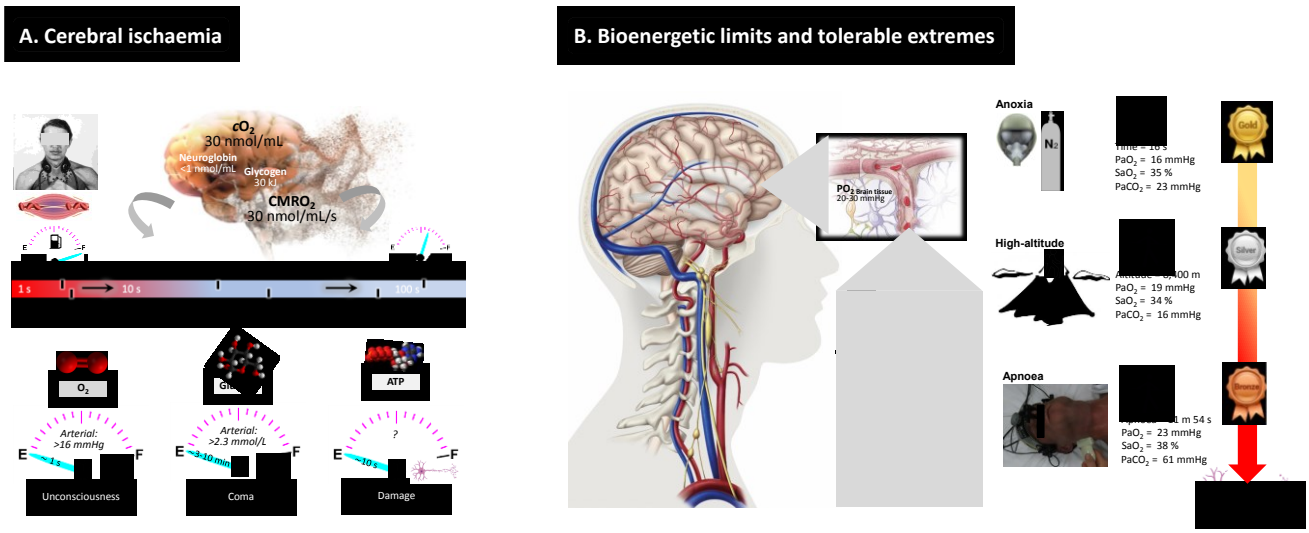

Figure 3. Walking the tightrope; pushing the human brain's bioenergetic limits

A. Excessive energy demands combined with limited oxygen (O_2)/glucose reserves renders the human brain especially sensitive to anoxia and ischaemia (top). This was highlighted during the (infamous) Red Wing studies involving inflation of a specialised cervical pressure cuff to induce cerebral ischaemia for up to 100 s in non-consenting psychiatric patients (see photo insert) (Rossen *et al.*, 1943). Note the onset and progression of neurological sequelae during prolonged ischaemia (middle) including theoretical limits of the human brain's energy reserves (bottom) based on the brain's bioenergetic demands (content and metabolic rate data presented in Figure 2B). B. Estimation of critical "tolerable limits" (figures in red) in the cerebral O_2 cascade thought to precede unconsciousness including the most hypoxaemic (notwithstanding hypo/hypercapnic) measurements recorded to date in humans (gold, silver and bronze awards according to severity of hypoxaemia) operating at the very limits of consciousness during laboratory [anoxia (Ernsting, 1963)/apnoea (Bailey *et al.*, 2017)] and field [terrestrial high-altitude (Grocott *et al.*, 2009)] experimentation. Note that the former experiment resulted in an arterial partial pressure of O_2 (PaO_2) of 16 mmHg yet ended in unconsciousness (as illustrated). These hypoxic challenges were accompanied by marked variations in the partial pressure(s) of carbon dioxide (16-61 mmHg) hence they are not "truly" comparable. Furthermore, regional/remote cerebral ischaemic preconditioning could, in theory, extend these tolerable limits. However, repeated and prolonged exposure to these environmental extremes may result in structural brain damage and long-term neurological complications. cO_2, oxygen content; $CMRO_2$, cerebral metabolic rate for O_2; PaO_2/CO_2, arterial partial pressure of O_2/carbon dioxide; N_2, nitrogen; SaO_2, arterial oxyhaemoglobin saturation.

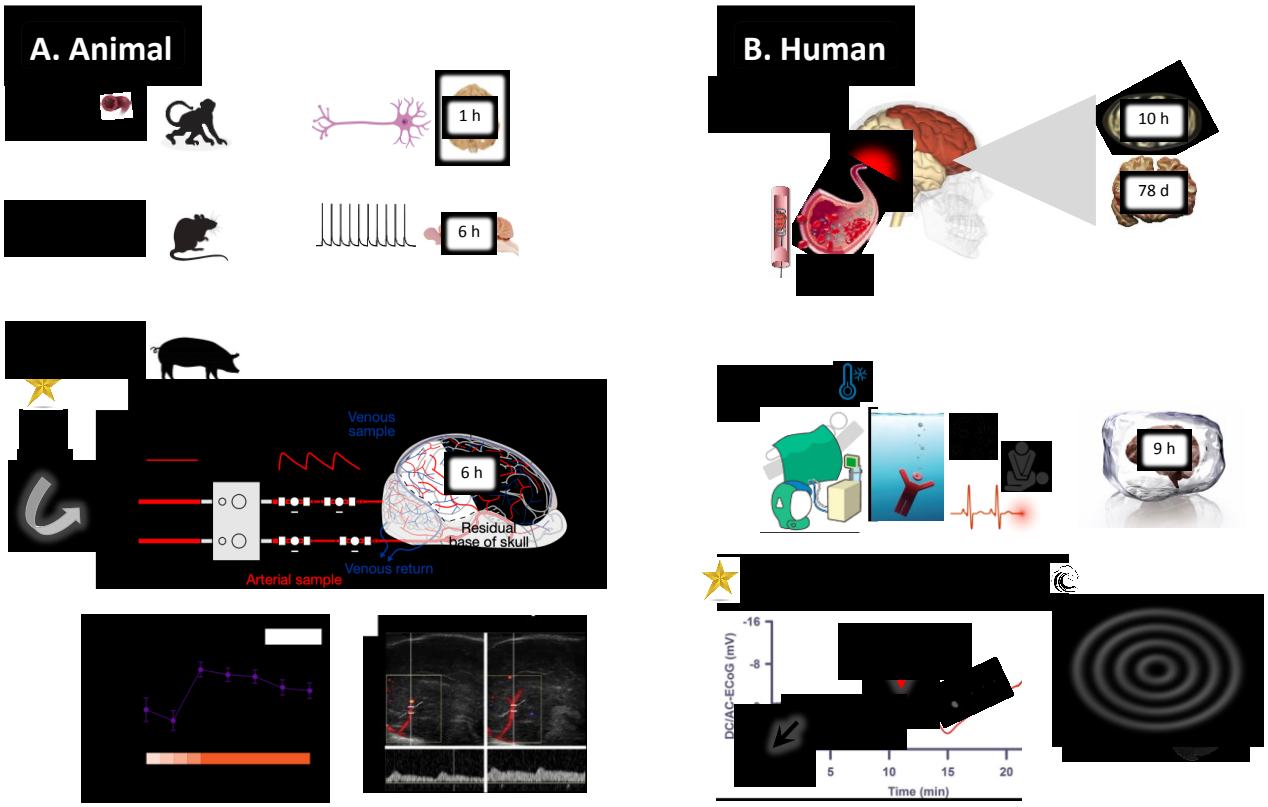

Figure 4. Beyond limits; the brain's unexpected resilience

The traditional notion that the mammalian brain is irreversibly damaged soon after blood stops circulating due to limited energy stores has been challenged by two landmark studies in particular ((Vrselja *et al.*, 2019) Y, both marked by a gold star), sparking widespread debate over the clinical diagnosis of brain death and organ transplantation. A. Neuronal, electrophysiological and metabolic recovery have been observed after global cerebral ischaemia or following a prolonged postmortem interval (PMI) in the animal brain. Bottom figures (marked by gold star) modified from (Vrselja *et al.*, 2019) illustrating cerebral metabolic rate for oxygen ($CMRO_2$) in 6–8-month-old pigs (*Sus scrofa domesticus*, *n* = 4) for up to 6 h after a 4 h postmortem interval using a customised extracorporeal pulsatile-perfusion system and haemoglobin-based, acellular, non-coagulative, echogenic, cytoprotective perfusate ("Brain*Ex*"), with arteriovenous gradients highlighting a remarkable resurgence of O_2 (and glucose, data now shown) consumption; colour doppler flow data also highlight increased flow in the pericallosal artery following administration of nimodipine, an *L*-type

voltage-gated calcium channel antagonist that increases cerebral perfusion, signifying retention of vascular dilatation; PG, pulse generator transforming continuous flow to pulsatile perfusion; L/R-ICA, left/right internal carotid artery; NS, non-significant ($P > 0.05$) and *significant ($P < 0.05$) using paired samples *t*-tests. B. Human brain tissue remains functional after a prolonged PMI (from 10 h in mitochondria to 78 d in brain slices) and favourable patient outcomes have been reported following endovascular thrombectomy for up to 16 h following ischaemic stroke. Hypothermia (both therapeutic and accidental due to drowning/cold exposure) can further extend the "living" brain's functional survival time. A recent study (Dreier *et al.*, 2018) has identified that the "dying" brain can linger in an isoelectric state following circulatory arrest for much longer than traditionally assumed. Bottom figure [marked by gold star modified from (Dreier *et al.*, 2018)] illustrates how intermittent waves (brain tsunamis) of spreading depolarisation (SD) eventually sweep through the cortex marking the loss of stored electrochemical energy and onset of neuronal damage thought to be more accurately forewarn impending brain death; DC/AC-ECog, direct current/alternate current-electrocorticography; mV, millivolts.

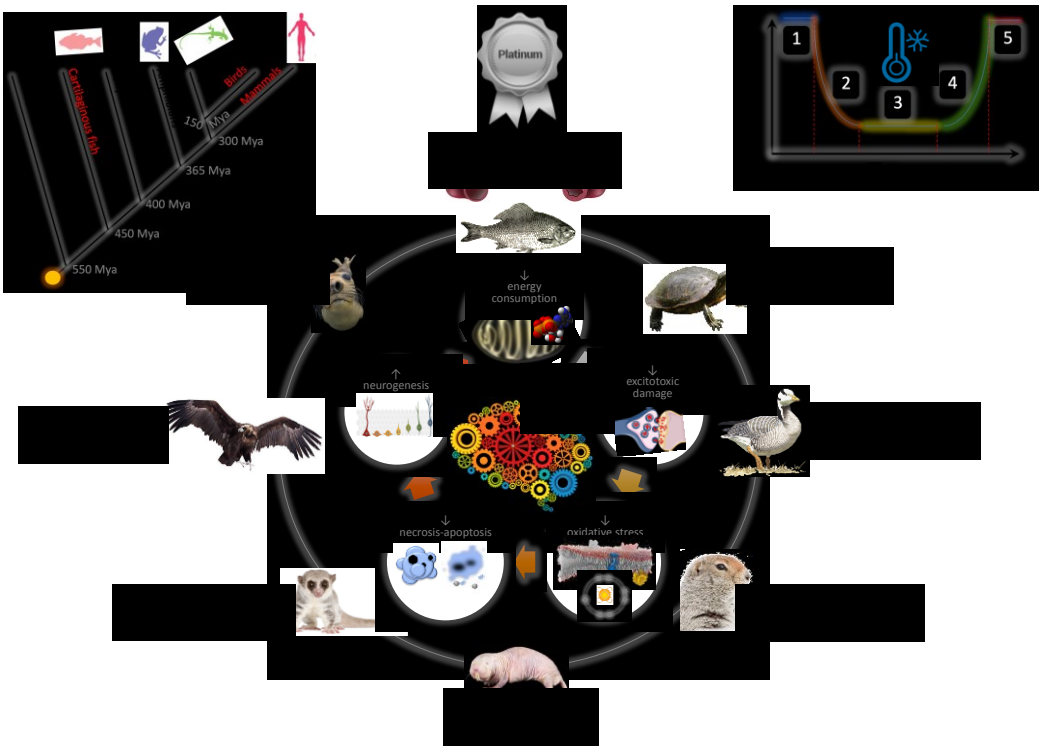

Figure 5. Nature's neurons; survival strategies adopted by anoxia-hypoxia tolerant vertebrates

Human tolerance to hypoxia, although impressive, falls well short of Nature's more resilient "extremophiles", including select species of swimmers (common crucian carp, *Carassius carassius*), freshwater turtle, red-eared pond slider, *Trachemys scripta*), divers (hooded seal, *Cystophora cristata*), burrowers (naked mole-rat, *Heterocephalus glaber*), hibernators (arctic ground squirrel, *Spermophilus parryii*, Eastern dwarf lemur, *Cheirogaleus major*) and flyers (Ruppell's griffon, *Gyps rueppellii*, bar-headed goose, *Anser indicus*) that reign supreme (platinum awards) with remarkable intrinsic tolerance to hypoxia-anoxia. The swimmers (carp and turtle) are arguably the most hypoxia-anoxia tolerant of all vertebrates, equipped with highly-specialised adaptations of brain physiology honed millions of years ago (Mya) over the course of evolution (upper left insert highlighting phylogenetic tree of vertebrate evolution that began ~550 Mya). Their survival strategies are diverse, broadly categorised into 4 phases [upper right insert, adapted from (Lutz & Milton, 2004)]:

1, constitutive factors including elevated levels of glycogen and antioxidants; 2, metabolic downregulation involving suppression of ATP turnover; 3, basal maintenance with ATP turnover dialled down to a minimum and 4, recovery, characterised by suppression of oxidative-inflammatory-nitrosative stress and apoptosis. The central schema highlights key adaptations that collectively function to preserve cerebral oxygen (O_2) homeostasis and cognition by increasing or at least maintaining cerebral blood flow, coupling cerebral O_2 (and glucose) delivery (cDO_2/cD_{Gluc}) to tissue metabolic demand [adapted from (Larson *et al.*, 2014)]. Better understanding of the neuroprotective strategies exploited by these specialists may help unlock some of the mysteries of the human brain including "hidden" O_2 stores/resilience and help identify new therapeutic targets for diseases characterised by O_2 deprivation, ischaemic/reperfusion injury and ageing (Nilsson & Lutz, 2004; Krivoruchko & Storey, 2010; Milton & Dawson-Scully, 2013; Larson *et al.*, 2014). Hibernation and cerebral hypometabolism have recently been documented in primates [dwarf lemurs, (Dausmann *et al.*, 2004; Blanco *et al.*, 2013)] with torpor not solely confined to cold-blooded mammals, indirectly implying that perhaps we humans may have the ability to hibernate that has beneficial applications from medicine to long-term space exploration (Chouker *et al.*, 2019). Several species take advantage of active brain cooling (upper right insert) to induce cerebral hypometabolism consistent with the Q_{10} effect, forming the basis for therapeutic hypothermia as a neuroprotective intervention in human patients after cardiac arrest/ischaemic stroke (Duan *et al.*, 2019).

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